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 NEWS  4  DEC 14   2006 MeSH terms loaded in MEDLINE/LMEDLINE
 NEWS  5  DEC 14   2006 MeSH terms loaded for MEDLINE file segment of TOXCENTER
 NEWS  6  DEC 14   CA/CAplus to be enhanced with updated IPC codes
 NEWS  7  DEC 21   IPC search and display fields enhanced in CA/CAplus with the
                   IPC reform
 NEWS  8  DEC 23   New IPC8 SEARCH, DISPLAY, and SELECT fields in USPATFULL/
                   USPAT2
 NEWS  9  JAN 13   IPC 8 searching in IFIPAT, IFIUDB, and IFICDB
 NEWS 10  JAN 13   New IPC 8 SEARCH, DISPLAY, and SELECT enhancements added to
                   INPADOC
 NEWS 11  JAN 17   Pre-1988 INPI data added to MARPAT
 NEWS 12  JAN 17   IPC 8 in the WPI family of databases including WPIFV
 NEWS 13  JAN 30   Saved answer limit increased
 NEWS 14  JAN 31   Monthly current-awareness alert (SDI) frequency
                   added to TULSA
 NEWS 15  FEB 21   STN AnaVist, Version 1.1, lets you share your STN AnaVist
                   visualization results
 NEWS 16  FEB 22   Status of current WO (PCT) information on STN
 NEWS 17  FEB 22   The IPC thesaurus added to additional patent databases on STN
 NEWS 18  FEB 22   Updates in EPFULL; IPC 8 enhancements added

 NEWS EXPRESS  FEBRUARY 15 CURRENT VERSION FOR WINDOWS IS V8.01a,
               CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 19 DECEMBER 2005.
               V8.0 AND V8.01 USERS CAN OBTAIN THE UPGRADE TO V8.01a AT
               http://download.cas.org/express/v8.0-Discover/

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FILE 'HOME' ENTERED AT 15:22:01 ON 27 FEB 2006
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=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 15:22:09 ON 27 FEB 2006

 FILE LAST UPDATED: 23 FEB 2006 (20060223/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
 on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
 See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> s hedgehog
          3649 HEDGEHOG
           852 HEDGEHOGS
L1         4017 HEDGEHOG
                (HEDGEHOG OR HEDGEHOGS)

=> s myocardial
        244527 MYOCARDIAL
             3 MYOCARDIALS
L2      244527 MYOCARDIAL
                (MYOCARDIAL OR MYOCARDIALS)

=> s l2 and l1
L3           12 L2 AND L1

=> s l3 not py>2000
       2934825 PY>2000
                (PY>20009999)
L4            9 L3 NOT PY>2000

=> d ibib 1-9

L4   ANSWER 1 OF 9     MEDLINE on STN
ACCESSION NUMBER:    2000465512     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 11021439
TITLE:               Cardiomyopathy in captive African hedgehogs
                     (Atelerix albiventris).
AUTHOR:              Raymond J T; Garner M M
CORPORATE SOURCE:    Northwest ZooPath, Snohomish, WA 98296-4815, USA.
SOURCE:              Journal of veterinary diagnostic investigation : official
                     publication of the American Association of Veterinary
                     Laboratory Diagnosticians, Inc, (2000 Sep) Vol. 12, No. 5,
                     pp. 468-72.
                     Journal code: 9011490. ISSN: 1040-6387.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       (CASE REPORTS)
                     Journal; Article; (JOURNAL ARTICLE)
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LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125

L4 ANSWER 2 OF 9 MEDLINE on STN
ACCESSION NUMBER: 96426701 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8828980
TITLE: How often has Lp(a) evolved?.
AUTHOR: Lawn R M
CORPORATE SOURCE: Falk Cardiovascular Research Center, Stanford University School of Medicine, CA 94305-5246, USA.
SOURCE: Clinical genetics, (1996 Apr) Vol. 49, No. 4, pp. 167-74.
Ref: 61
Journal code: 0253664. ISSN: 0009-9163.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961210

L4 ANSWER 3 OF 9 MEDLINE on STN
ACCESSION NUMBER: 94273536 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8004994
TITLE: Microcalorimetric study on myocardial metabolism in a hibernator and two nonhibernators at 20 degrees C and 37 degrees C.
AUTHOR: Ikomi-Kumm J; Monti M; Hanson A; Johansson B W
CORPORATE SOURCE: Department of Internal Medicine, Lund University Hospital, Malmo, Sweden.
SOURCE: Cryobiology, (1994 Apr) Vol. 31, No. 2, pp. 133-43.
Journal code: 0006252. ISSN: 0011-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940729
Last Updated on STN: 19940729
Entered Medline: 19940715

L4 ANSWER 4 OF 9 MEDLINE on STN
ACCESSION NUMBER: 91138357 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2286096
TITLE: Mechanical restitution at different temperatures in papillary muscles from rabbit, rat, and hedgehog.
AUTHOR: Liu B; Wohlfart B; Johansson B W
CORPORATE SOURCE: Department of Pharmacology, University of Lund, Sweden.
SOURCE: Cryobiology, (1990 Dec) Vol. 27, No. 6, pp. 596-604.
Journal code: 0006252. ISSN: 0011-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910412
Last Updated on STN: 19910412
Entered Medline: 19910326

L4 ANSWER 5 OF 9 MEDLINE on STN
ACCESSION NUMBER: 91065005 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2249456
TITLE: Effects of low temperature on contraction in papillary muscles from rabbit, rat, and hedgehog.
AUTHOR: Liu B; Wohlfart B; Johansson B W
CORPORATE SOURCE: Department of Pharmacology, University of Lund, Sweden.
SOURCE: Cryobiology, (1990 Oct) Vol. 27, No. 5, pp. 539-46.
Journal code: 0006252. ISSN: 0011-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199101
ENTRY DATE: Entered STN: 19910308
Last Updated on STN: 19910308
Entered Medline: 19910115

L4 ANSWER 6 OF 9 MEDLINE on STN
ACCESSION NUMBER: 87029426 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3769518
TITLE: Effects of induced hypothermia on organ blood flow in a hibernator and a nonhibernator.
AUTHOR: Sjoquist P O; Duker G; Johansson B W
SOURCE: Cryobiology, (1986 Oct) Vol. 23, No. 5, pp. 440-6.
Journal code: 0006252. ISSN: 0011-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198611
ENTRY DATE: Entered STN: 19900302
Last Updated on STN: 19900302
Entered Medline: 19861125

L4 ANSWER 7 OF 9 MEDLINE on STN
ACCESSION NUMBER: 86108432 MEDLINE
DOCUMENT NUMBER: PubMed ID: 4085517
TITLE: Ventricular repolarization and fibrillation threshold in hibernating species.
AUTHOR: Johansson B W
SOURCE: European heart journal, (1985 Nov) Vol. 6 Suppl D, pp. 53-62.
Journal code: 8006263. ISSN: 0195-668X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: (CASE REPORTS)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198603
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860311

L4 ANSWER 8 OF 9 MEDLINE on STN
ACCESSION NUMBER: 85100400 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6518802
TITLE: Cardiac responses in relation to heart size.
AUTHOR: Johansson B W
SOURCE: Cryobiology, (1984 Dec) Vol. 21, No. 6, pp. 627-36.
Journal code: 0006252. ISSN: 0011-2240.
Report No.: NASA-85100400.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

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FILE SEGMENT:       Priority Journals; Space Life Sciences
ENTRY MONTH:        198502
ENTRY DATE:         Entered STN: 19900320
                    Last Updated on STN: 19900320
                    Entered Medline: 19850226

L4   ANSWER 9 OF 9     MEDLINE on STN
ACCESSION NUMBER:   62045823     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 13904450
TITLE:              Myocardial lactate concentration in guinea-pigs,
                    normothermic and hypothermic, and hedgehogs, in a
                    hibernating and a non-hibernating state.
AUTHOR:             HANSON A; JOHANSSON B W
SOURCE:             Acta physiologica Scandinavica, (1961 Oct) Vol. 53, pp.
                    137-41.
                    Journal code: 0370362. ISSN: 0001-6772.
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       OLDMEDLINE; NONMEDLINE
ENTRY MONTH:        199811
ENTRY DATE:         Entered STN: 19990716
                    Last Updated on STN: 19990716
                    Entered Medline: 19981101


=> file pctfull
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     2.31       2.52

FILE 'PCTFULL' ENTERED AT 15:23:24 ON 27 FEB 2006
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FILE LAST UPDATED:          21 FEB 2006      <20060221/UPTX>
MOST RECENT UPDATE WEEK:         200607
FILE COVERS 1978 TO DATE

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>>> SDI SEARCHES (ALERTS) WILL BE RESUMED WHEN BIBLIOGRAPHIC DATA
    BECOME AVAILABLE <<<

=> s hedgehog
          1002 HEDGEHOG
            55 HEDGEHOGS
L5        1029 HEDGEHOG
                 (HEDGEHOG OR HEDGEHOGS)

=> s myocardial
L6       17861 MYOCARDIAL

=> s l6 and l5
L7         165 L6 AND L5

=> s structure or formula or compound
```

```
        418360 STRUCTURE
        206597 STRUCTURES
        455983 STRUCTURE
                 (STRUCTURE OR STRUCTURES)
        151185 FORMULA
         24694 FORMULAS
         25119 FORMULAE
        158696 FORMULA
                 (FORMULA OR FORMULAS OR FORMULAE)
        204205 COMPOUND
        215366 COMPOUNDS
        263248 COMPOUND
                 (COMPOUND OR COMPOUNDS)
L8      578073 STRUCTURE OR FORMULA OR COMPOUND

=> s l8 and l7
L9         163 L8 AND L7

=> s l9 not py>1999
        630082 PY>1999
L10         18 L9 NOT PY>1999

=> s agonist
         25066 AGONIST
         27468 AGONISTS
L11      34707 AGONIST
                 (AGONIST OR AGONISTS)

=> s l11 and l10
L12          6 L11 AND L10

=> d ibib 1-6

L12    ANSWER 1 OF 6     PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:       2006009836 PCTFULL
       no bibliographic data available - please use FPI for PI information
DESIGNATED STATES

L12    ANSWER 2 OF 6     PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:       2006008342 PCTFULL
       no bibliographic data available - please use FPI for PI information
DESIGNATED STATES

L12    ANSWER 3 OF 6     PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:       2006006948 PCTFULL
       no bibliographic data available - please use FPI for PI information
DESIGNATED STATES

L12    ANSWER 4 OF 6     PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:       1999064627 PCTFULL  ED 20020515
TITLE (ENGLISH):        PROBES USED FOR GENETIC FILING
TITLE (FRENCH):         SONDES UTILISEES POUR PROFILAGE GENETIQUE
INVENTOR(S):            ROBERTS, Gareth, Wyn
PATENT ASSIGNEE(S):     GENOSTIC PHARMA LIMITED;
                        ROBERTS, Gareth, Wyn
LANGUAGE OF PUBL.:      English
DOCUMENT TYPE:          Patent
PATENT INFORMATION:
                        NUMBER              KIND     DATE
                        ---------------------------------
                        WO 9964627            A2 19991216
DESIGNATED STATES
       W:               AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK
                        EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP
                        KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL
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PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN
YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ
MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU
MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD
TG

APPLICATION INFO.: WO 1999-GB1780 A 19990604
PRIORITY INFO.: GB 1998-9812099.1 19980606
GB 1998-9813291.3 19980620
GB 1998-9813611.2 19980624
GB 1998-9813835.7 19980627
GB 1998-9814110.4 19980701
GB 1998-9814580.8 19980707
GB 1998-9815438.8 19980716
GB 1998-9815576.5 19980718
GB 1998-9815574.0 19980718
GB 1998-9816085.6 19980724
GB 1998-9816086.4 19980724
GB 1998-9816921.2 19980805
GB 1998-9817097.0 19980807
GB 1998-9817200.0 19980808
GB 1998-9817632.4 19980814
GB 1998-9817943.5 19980819

L12 ANSWER 5 OF 6 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1999056785 PCTFULL ED 20020515
TITLE (ENGLISH): MUSCLE-DERIVED CELL MEDIATED GENE DELIVERY FOR TREATING MUSCLE- AND BONE-RELATED INJURY OR DYSFUNCTION
TITLE (FRENCH): TRANSPORT DE GENE EFFECTUE PAR L'INTERMEDIAIRE D'UNE CELLULE DE MUSCLE PERMETTANT DE TRAITER LES LESIONS OU LES DYSFONCTIONS MUSCULAIRES OU OSSEUSES
INVENTOR(S): CHANCELLOR, Michael, B.;
HUARD, Johnny
PATENT ASSIGNEE(S): UNIVERSITY OF PITTSBURGH
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9956785	A2	19991111

DESIGNATED STATES
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK
EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP
KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL
PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU
ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD
RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC
NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

APPLICATION INFO.: WO 1999-US9451 A 19990430
PRIORITY INFO.: US 1998-60/083,917 19980501

L12 ANSWER 6 OF 6 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1998035020 PCTFULL ED 20020514
TITLE (ENGLISH): METHODS FOR MODULATING HEMATOPOIESIS AND VASCULAR GROWTH
TITLE (FRENCH): PROCEDES DESTINES A MODULER L'HEMATOPOIESE ET LA CROISSANCE VASCULAIRE
INVENTOR(S): BARON, Margaret, H.;
FARRINGTON, Sarah, M.;
BELAOUSSOFF, Maria
PATENT ASSIGNEE(S): THE PRESIDENTS AND FELLOWS OF HARVARD COLLEGE
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE

	WO 9835020	A2 19980813
DESIGNATED STATES		
W:	CA JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE	
APPLICATION INFO.:	WO 1998-US2633	A 19980210
PRIORITY INFO.:	US 1997-60/037,513	19970210
	US 1997-60/049,763	19970616

=> d kwic 6

L12 ANSWER 6 OF 6 PCTFULL COPYRIGHT 2006 Univentio on STN

ABEN Methods and assays are provided for selecting compounds that are functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue for use in modulating hematopoiesis and vascular growth, such compound being exemplified by a hedgehog protein, and an agonist of a hedgehog protein binding receptor. According to the method, such compound causes undifferentiated mesodermally derived cells to undergo at least one of hematopoiesis or vasculogenesis. Examples of undifferentiated mesodermally derived cells. . .

ABFR . . . d'un embryon. Ces procedes sont destines a moduler l'hematopoiese et la croissance vasculaire, le compose etant notamment une proteine a structure dite en herisson, ainsi qu'un agoniste d'un recepteur de liaison de proteine a structure dite en herisson. Conformement a ce procede, un tel compose permet de soumettre a hematopoiese ou developpement du systeme vasculaire. .

DETD . . . mesodermally derived cells, to undergo at least one of hematopoiesis and vascular growth. The method includes the steps of selecting a compound that is functionally equivalent to a gene product expressed in an embryo's extraernbryonic tissue; and causing the compound to access the cells, so as to stimulate the cells to undergo at least one of hernatopoiesis and vascular growth.

. . . in vascular growth or hernatopoiesis in an embryo in utero, that includes the steps of: selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and causing the compound to access a population of embryonic cells in vivo, so as to stimulate the cells to undergo at least one of hematopoiesis. . .

. . . treating a subject suffering from an abnormal number of erythroid cells, that includes the steps of selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and causing the compound to access a population of hernatopoietic stem cells over an effective time so as to modulate the number of cells undergoing. . .

. . . for treating a subject suffering from an ischemia in tissues containing inesoderinally derived cells, that includes

selecting an effective dose of a compound that is functionally equivalent to a gene product
expressed in an extraembryonic tissue; and administering the compound to the ischemic site
over an effective time so as to stimulate vascular growth.

In another embodiment of the invention, an in vitro assay is provided for determining
the activity of a compound capable of modulating
hernatopoiesis or vascular growth, that
includes the steps of selecting a population of cells from a tissue derived. . .

In another embodiment of the invention, an assay is provided for determining the
activity of a compound capable of modulating hematopoiesis or vascular growth, that
includes the steps of selecting a first transgenic animal carrying a marker:E-globin hybrid
1. . . an embryo
from the mating at a time within the first third of the gestation period; and determining the
effect of the compound on the stimulation of hematopoiesis and vascular growth in the
isolated embryo by measuring marker expression.

Fig. 3 shows the formation of yolk sac-like structures by cultured blastocysts (a)
transgenic blastocysts prior to culture (b) Sac-like structure (non transgenic) stained with
benzidine to reveal hemoglobin containing cells (c) Sac from cultured transgenic blastocysts
stained with XGal to reveal hemoglobin. . .

Fig. 4 shows RT-PCR analysis of blastocyst cultures: (A) e-globin was observed in
blastocysts that have developed into sac-like structures (sac) but not in samples that were
relatively flat mounds of cells (flat). The higher molecular weight band is the internal
control-actin.. . .
.
but is absent in epiblasts only, as determined by XGal staining. Dashed lines were drawn around the epiblasts to facilitate visualization of
structures. (a) whole embryo on a filter; (b) epiblast on a filter; (c) whole embryo on a slide;
and (d) epiblast on. . .

Fig. 9 shows that recombinant hedgehog protein can substitute for visceral endoderm
to stimulate primitive hernatopoiesis in cultured epiblasts. Isolated epiblasts were cultured in
the absence (lanes labeled none) or presence of three different concentrations of
recombinant hedgehog protein (0.25, 1 and 5 Vg/ml). Primitive hernatopoiesis was assessed
by RT-PCR analysis for e-globin expression. Actin served as an internal.
. .

The circular structure represents a blastocyst of around 3.5 days.
.
stem cells and progenitor cells from embryo or adult. Embodiments of the invention are further directed to novel assays for identifying

compounds capable of
stimulating hernatopoiesis and vascular growth. Support for the methods of the invention are
provided in the examples contained herein. According to an embodiment of the invention,
compounds have been identified that are capable of stimulating blood development in the
embryo and in the adult and are functionally equivalent to gene products expressed in the
visceral endoderm and yolk sac mesoderm. Such gene products are exemplified by hedgehog
compounds, TGF-P, TNF, and WNT compounds and are here identified as achieving a
similar effect to that observed with extraembryonic tissues with regard to hematopoiesis and
vascular growth in undifferentiated mesodermal derived tissues. In an embodiment of the
invention, compounds including those selected from hedgehog and TGF-P may act
synergistically so as to enhance their stimulatory effect on target cells.

Synergistic effect is defined here as for two or more compounds where little or no
biological effect is observed with the compounds alone but together the compounds have a
potent biological effect.

Hedgehog compound is defined here and in the claims as a class of molecules of the
hedgehog family that includes recombinant hedgehog protein, analogs, and derivatives of
hedgehog proteins, and agonists and antagonists of hedgehog protein receptors and functional
equivalents of the aforementioned.

.
and
in the adult. According to embodiments of the invention, processes of vascular growth and
hematopoiesis in embryonic development are affected by compounds in the visceral
endoderm. For example, we have identified for the first time that hedgehog proteins act on
undifferentiated mesodermal derived cells in vitro to stimulate blood formation and on
embryonic tissue and yolk sac development at very early stages in the hernatopoiesis and
vascular growth pathways. Furthermore, according to the invention, these early acting
compounds have utility in regulating hematopoiesis and vascular growth in the adult animal.

.
addition of visceral endoderm which is sufficient to cause the anterior epiblast to
form blood islands. When either visceral endoderm or hedgehog protein was added to the
culture, blood formation was observed. (Figure 16)
(iv) Explants or embryoid bodies derived from mutants defective in.

.
visceral endoderm such that its absence results in the failure to make blood, is a
suitable model system for screening novel compounds from libraries such as those derived
from extraembryonic tissues, where these libraries include combinatorial

peptide libraries and
recombinant DNA libraries. By using a pooling strategy to reduce the number of
experimental tests, compounds may be identified that are
useful in modulating hematopoiesis
and vascular growth in embryoid bodies.

type of assay can be used to study the effect of other mutations, such as
deficiency of signaling factors such as hedgehog proteins (for example, Indian hedgehog), on
blood formation.(Examples 3-5) For example, Ihh null mutant ES cells may be formed and
factors capable of overcoming the mutation, identified.. These cells could be rescued either
by providing exogenous hedgehog protein or by transfecting the cells with vectors expressing
a hedgehog gene utilizing standard vectors or retroviral vectors.(Figure 9) The mutated cells
could also be reintroduced into mice to form chimeras.

assay for expression of many
genes from a single culture product.(Figure 4)
Using the above assays, we have identified a number of compounds that are
functionally equivalent to gene products that are expressed in extraembryonic tissues and
may stimulate blood fori-nation. These compounds include TGF-P proteins more specifically
TGF-P I more specifically bone morphogenic protein (BMP) more specifically BMP-4; tumor
necrosis factor (TNF) proteins more specifically TNF-a; wnt family; and hedgehog proteins.

(Figures 5,9 and 17) Compounds may also include naturally occurring and synthetic agonists,
antagonists, analogs and derivatives of the above. These molecules may interact with
membrane proteins which initiate signal transduction pathways resulting in a biological
response. Therefore, in addition to the above compounds, agonists and antagonists to these
membrane binding proteins including those receptors, receptor agonists and receptor
antagonists associated with hedgehog binding receptors and hedgehog signalling transduction
pathways such as smoothened, patched and gli may have utility in regulating hernatopoiesis
and vascular growth.

G) screening libraries of compounds for activity in stimulating hernatopoiesis and
vascular growth;
(ii) testing for the effect of growth factors, cytokines and other signaling
molecules on embryonic hernatopoiesis and also on vascular growth;
(iii) determining the effect of hedgehog proteins on hematopoiesis and vascular
growth in the embryo, fetus and adult. For example, the blastocyst assay may be used to
determine the effect of hedgehog proteins on yolk sac' development ex vivo where the
blastocyst is derived from transgenic or non-transgenic animals.

mesoderm is of the same

origin as that of the yolk sac;
(v) following the development of primitive erythroid cells and vascular structures
by staining with a marker such as XGal so as to outline the vasculature and permit the
tracking of vascular growth as. . . individual explants of targeted mutations in genes
that affect hematopoiesis or vascular growth in the parent animal including those carrying
transgenes expressing hedgehog, patched, Gli and other proteins; and
(vii) examining the effect of gene therapy on mesodermally derived tissues; where
for example, the gene for hedgehog protein is introduced into prestreak embryos deprived of
the visceral endoderm, under various promoters so as to modulate the effect of. . .

Hedgehog proteins: We have shown here for the first time that hedgehog proteins are
capable of stimulating hernatopoiesis in the yolk sac, and the splanchnopleura and other
hematopoietic tissues of the embryo or fetus. . . of the adult.(Examples 3-5, Tables 1-2, Figs 6,9). By screening for molecules that
were present in the visceral endoderm, we identified hedgehog gene product. When a
hedgehog protein (SHH) was added to epiblast cultures and RNA was isolated after 2-3 days
and analyzed by RT-PCR (Example 3, Fig.. . .

The above assays show that hedgehog proteins expressed in extraembryonic tissue as
well as hedgehog proteins that are closely related to proteins expressed in extraernbryonic
tissues, stimulate hernatopoiesis and vasculogenesis. Members of the hedgehog family which
are a distinct family of signaling molecules (e.g., reviewed in Goodrich et al.,Genes &
Develop. 10 (1996), 301-12) are known. . . spermatogenesis. The family was initially identified as
involved in normal segmental patterning in Drosophila (Nusslein-Volhard et al, Nature, 287
(1980), 795-801). The hedgehog family includes Desert hedgehog (DHH) protein, Indian
hedgehog protein (IHH), Moonrat hedgehog (Zebrafish) and Tiggy winkle hedgehog (Zebra
fish).

The utility of the hedgehog proteins in stimulating hematopoiesis and vascular growth
is further reinforced by our experiments on target molecules through which these proteins act.

In support of our observations that hedgehog proteins are capable of stimulating
hematopoiesis, we identified the enriched expression of Gli and patched in yolk sac
mesoderm. Gli is a transcription factor involved in the transduction pathway on which
hedgehog proteins act, while PTC (patched) is a membrane protein that binds hedgehog
protein to initiate the signal transduction pathway that ultimately causes a biological response
in the target cell. The association of these proteins with yolk sac

mesoderm further supports
the observation that hedgehog proteins stimulate
hematopoiesis. Since ptc is the presumed
gateway to a cell response, any agonist of hedgehog
capable of binding patch is expected to
induce the same biological effect as hedgehog-in this case,
hematopoiesis and vascular
growth.

Certain hedgehog proteins have been reported to be involved in
the initiation of
expression of the secondary signaling molecules-BMP-2 and BMP-4
(proteins belonging. . . to
the TGF-P family) in the mesoderm and Fgf-4 in the ectoderm (WO
95/18856). We have
identified for the first time, that hedgehog proteins might
interact in a synergistic manner with
secondary signaling molecules to stimulate hematopoiesis and vascular
growth (Example 6).

The activity of compounds that are functional equivalents to a
gene product expressed
in extra-embryonic tissue such as recombinant hedgehog
protein, analogs, derivatives and
dissociation products of hedgehog proteins, and
agonists of hedgehog protein receptors such
as PTC according to the invention, may stimulate hematopoiesis and
vascular growth by
1 5 acting on cells or. . .

The invention includes the use of functional peptides of
hedgehog protein. The term
functional peptide as a subclass of a hedgehog
compound defined above, is meant to
include peptide fragments of the hedgehog protein that are
capable of inducing a biological
activity that is the same or equivalent to the entire protein (WO
96/16668, incorporated here
by reference). The invention further includes hedgehog
compounds described in WO
95/18856 and here incorporated by reference, including homologs of
hedgehog proteins,
recombinant hedgehog proteins, hedgehog encoding
nucleic acids, antisense molecules, gene
constructs for use in gene therapy including viral vectors known in the
art, combinatorial
mutants of hedgehog proteins as agonists or
antagonists, and antibodies specific for hedgehog
protein epitope. These and other compounds may be selected for
modulating hernatopoiesis
and vascular growth according to the assays of the invention.

invention, these factors may be used to stimulate hematopoiesis and
vascular growth in animals including mammals, including humans.
Similarly antagonists to
the compounds of the invention may be used to inhibit vascular
growth and hematopoiesis.

Our novel blastocyst assay may be used to determine the effect of
hedgehog proteins
on yolk sac development. In addition, blastosacs could be assayed for
gene expression not
only using LacZ as a histochemical marker,. . .

Transgenic mouse models for studying the effect of selected

compounds on hematopoiesis
and vascular growth.

. . .

al. J.Biol. Chem. Vol 270, (1995) pp 1289-1294). Other transgenic mice may be formed in
which a selected sequence from the hedgehog gene family may be placed under control of an
enhancer and/or promoter of the sort described above. Furthermore, transgenic mice may be
generated in which the hedgehog or hedgehog
agonist or antagonist is expressed under the
control of heterologous tissue specific promoters/enhancers such as described above. Other
transgenic animals may be formed in which hedgehog regulatory sequences are used to drive
expression of heterologous gene coding sequences in specific embryonic or adult tissues eg
Ihh regulatory sequences. . .

Science vol 269 (1995)pp 679-682, to target hedgehog genes
into selected sites in the
genome under the control of endogenous sequences in embryonic stem (ES) cells. These
modified ES cells. . .

. . .

to blood diseases such as leukemias, and abnormal vascular growth and abnormal
hernatopoiesis. These events may be analyzed with regard to
hedgehog compounds.

There are a number of therapeutic applications for compounds
of the invention. Such
uses are associated with the modulation of hematopoiesis and vascular growth and include
methods that result in stimulation as well as those that result in inhibition of proliferation
and/or differentiation of stem cells. Examples of compounds of
the invention have been
discussed above.

(a) therapeutic compounds such as hedgehog proteins
including derivatives,
analogs, and degradation products of naturally occurring proteins; agonists or antagonists of
protein receptors as well as functional equivalents of the above listed compounds. The
therapeutic compounds may be isolated from cultures of
extra-embryonic tissues,
manufactured by recombinant technology or prepared by synthetic
chemistry;
(b) coding sequences for the above- listed therapeutic compounds
, incorporated
into vectors suited for gene therapy techniques; and
(C) mammalian cells that have been transformed with coding sequences of the
above for. . .

. . .

of the techniques available
in the art. For example, a protein, analogue, derivative, antagonist or receptor, of an
identified protein (collectively called compounds) such as
hedghog related compounds, may
be introduced into a vector and the vector introduced into the appropriate target tissue where
this tissue is located in an. . . enhancer to ensure selective

expression in the targeted tissue. For
example, use of the cardiac actin enhancer to express the desired
compound in the heart, the
MCK enhancer to express the compound in skeletal muscle; sca-I
regulatory sequences to
1 5 express hedgehog compound in hematopoietic stem
cells or a retina-specific regulatory
element of the interphotoreceptor retinoid-binding protein to express
the compound in the
retina.

. . .

heterologous cells contained within an immune protective barrier, may be
manipulated by standard techniques to secrete the selected protein such
as hedgehog, or
analogues, derivatives, antagonists or receptors of protein.

. . .

lineages. Examples of targets for such treatments include in vivo or in
vitro exposure of undifferentiated mesodermally derived cells to a
compound of the
invention. Examples of target cells include bone marrow stem cells,
progenitor cells, and
cord blood cells. These cells may be. . . or the cells may be freshly
isolated and maintained in vitro in a culture
medium., Exposure of such cells to the compound results in
enhanced proliferation and/or
differentiation of the cells, the stimulated cells being implanted in
the same or different
subject from which. . .

. . .

from disease caused by
infectious agents such as human immune deficiency virus and may be
treated using a method
1 0 and compounds that stimulate hematopoiesis. The
consequences of such abnormalities if
untreated are various forms of anemia (associated with abnormally low
levels of
erythrocytes).. . .

. . .

degenerative disease, aging, trauma, or infectious agents. Examples
include
diabetic chronic ulcers, bums, frost bite, ischernic events following
stroke and
transplantation. The compounds of the invention may be used in
the adult for induction of
revascularization or formation of collateral vessels in ischemic
myocardium or ischemic
limbs, and in coronary artery bypasses and in promoting wound healing in
general. For
example, compounds of the invention may be used in treatment
of duodenal ulcers by
enhancing microvessel density and promoting more rapid healing. In. .

. . .

5'-ACACGATGCCATGCTGGTCA-3'
c-myosin(5') 5'-CTCGCAGAACAGCAGCCTAA-3' PCR product is 679bp; 32 cycles
c-myosin(3') 5-AGGGTCTGCTGGAGAGGTTA-3'
(C) BLASTOCYSTS ISOLATED AT ABOUT 3 3.5DPC PROVIDE A
MODEL SYSTEM FOR SCREENING COMPOUNDS THAT CAN
STIMULATE HEMATOPOIESIS AND VASCULAR GROWTH OF
UNDIFFERENTIATED MESODERMAL CELLS
Blastocyst cultures were prepared and used to analyze the effects of
compounds on
the stimulation of undifferentiated mesodermal derived cells to undergo
hernatopoiesis and
vasculogenesis. The blastocyst culture system described here is suited

for following the
development of embryonic structures in vitro, such as the yolk
sac, that normally form post
implantation in vivo. The effects of exogenously added growth factors.
. .
.
(2,000 U/ml),
streptomycin (2,000 pg/ml), 2 niM glutamine, I mM pyruvate, 0. I mM
nonessential amino
acids (GIBCO-BRL), and 10-4M P-mercaptoethanol. Sac-like
structures could first be seen
around 7 days in culture; by 9- 1 0 days they had enlarged to the point
where they were easily
visible with the naked eye (0 2 mm in diameter). These sac-like
structures (here termed
blastosacs) closely resembled early inurine yolk sacs.

4A, embryonic globin is produced only when yolk sac-like
structures form, but not if the
blastocysts do not progress in their development beyond an amorphous
mound of
trophectoderm cells.

Null mutant emblyoid bodies Embryoid bodies are structures
derived from ES cells
that form blood islands under appropriate culture conditions (Keller
(1995)). We have
developed an assay system using embryoid. . . 195) Gene Targeting: A
Practical Approach (New York: IRL Press). with mutations in selected
genes were rescued
by addition of a compound that is functionally equivalent to
the gene product expressed by
the non- mutated gene.

Example 3: Compounds that are functionally equivalent to a
gene product
expressed in an embryo's extraembryonic tissue (exemplified by
hedgehog protein) stimulate hematopoiesis and vascular growth
of
undifferentiated mesodermal cells (exemplified by epiblast
mesoderm)
(a) A hedgehog protein, typified by Sonic hedgehog,
was demonstrated to
stimulate hernatopoiesis in the epiblast mesoderm using the method of
Example 2(A) (Fig. 9).

(b) Compounds that are functionally equivalent to a gene
product expressed in an
embryo's extraernbryonic tissue (exemplified by hedgehog
protein) stimulate hematopoiesis
and vascular growth of undifferentiated mesodermal cells (exemplified by
adult bone marrow
cells).

To determine whether recombinant hedgehog proteins influence
the development or
differentiation of adult hematopoietic stem or progenitor cells, we
carried out in vitro clonal
assays. Mononuclear cells. . .
.
bovine serum albumin (cell culture grade BSA, 1%), 2-
mercaptoethanol (I x I OM) and the indicated growth factors and
recombinant hedgehog
proteins. Recombinant human erythropoietin (Epo) was obtained from Amgen
and used at 40

U/nil. Recombinant interleukin-3 (IL-3) and granulocyte/macrophage-colony stimulating
factor (GM-CSF) were. . . were scored on the days indicted. Colonies were scored as CFU-E, BFU-E, myeloid
or mixed. Where included in the cultures, recombinant hedgehog proteins were added at
concentrations between I and 5 yg/ml. Buffer alone (5 mM sodium phosphate pH 5.5
150mM NaCl, 0.5 mM. . .

. . .

all types (erythroid: CFU-E,
BFU-E; myeloid: CFU-GM) were increased by - 1. 5 to more than 4-fold, in a dose-dependent
manner (recombinant hedgehog protein added at 1, 2.5, 5yg/ml,
X ug). The observation that
hedgehog proteins are apparently not selective for erythroid versus myeloid lineage is
consistent with the hypothesis that they stimulate stem or early. . .

All three recombinant hedgehog proteins stimulated colony formation. From these data we
conclude that both SHH and IHH enhance proliferation, differentiation and/or survival of
hematopoietic stem/progenitor. . .

. . .

were stored in buffer pH 8.0; untagged SHH was stored in buffer pH 5.
Other approaches to measuring the effect of compounds that are functionally
equivalent to a gene product expressed in an embEyo's extraembryonic tissue on
undifferentiated mesodermal cells.

. . .

by flow cytometry (florescence-activated cell sorting,
FACS) or magnetic immunoselection (Testa and Molineux, 1993) and their development
enhanced in the presence of hedgehog protein. These resulting populations are examined
using in vivo assays include the CFU`-S assay (spleen colony-forming unit) and long-term
bone marrow cultures. . .

. . .

sac mesoderm. (Fig. 6) The enriched expression
of Gli and patched in yolk sac mesoderm points to mesoderm as target of hedgehog
signalling.: Yolk sacs from 10.5 and 12.5 dpc embryos were separated into endoderm (e) and
mesoderrn (m) fractions and RNA was prepared. . .

Example 6: Synergistic effect of Hedgehog protein with TGF-P proteins on
1 5 hematopoiesis (and vascular growth)
Using the methods of Example 3(A) above, we have shown using RT-PCR, that both
Indian Hedgehog and BMP-6 are expressed in early visceral endoderm. Whole embryo
(6.5dpc), epiblasts, epiblasts plus hedgehog protein,
epiblasts plus BMP-6 protein and
epiblasts plus hedgehog protein and BMP-6; are examined after 72 hrs incubation to
determine the extent of activation Of E-globin expression. The experiment is repeated for
BMP-2, BMP-4 and BMP We expect to observe an enhanced effect when both hedgehog
and BMP-4 are present compared with either alone.

CLMEN. . . stimulating a population of undifferentiated mesodermally derived cells to undergo at least one of hematopoiesis and vascular growth; comprising:
(a) selecting a compound that is functionally equivalent to a gene product
expressed in an embryo's extraembryonic tissue;
(b) causing the compound to access the cells, so as to stimulate the cells to
undergo at least one hernatopoiesis and vascular growth.

2 A method according to claim 1, wherein the compound is a secreted protein.

3 A method according to claim 1, wherein the compound is a hedgehog
compound.

4 A method according to claim 3, wherein the compound is an agonist of a
hedgehog protein binding receptor.

5 A method according to claim 4, wherein the hedgehog protein binding receptor
is patched.

6 A method according to claim 1, wherein the compound causes enriched
expression of Gli.

7 A method according to claim 3, wherein the hedgehog compound is selected
from the group consisting of Indian hedgehog, Desert hedgehog and Sonic hedgehog
compound.

8 A method according to claim 3, wherein the compound is an Indian hedgehog
compound,

9 A method according to claim 1, wherein the compound is a first compound
derived from a first gene product and is capable of acting synergistically with a second
compound that is derived from a second gene product expressed in the extraembryonic tissue,
so as to enhance the stimulation of at. . .

10 A method according to claim 9, wherein the second compound is a functional
equivalent of a TGF-P family member.

. . .
further comprising the step of maintaining the
cell population in vitro in a culture medium such that step (b) includes providing the
compound in the culture medium.

. . .
to claim 14, wherein the cells are precursor cells from an adult human capable of vascular growth when stimulated by the compound.

25 A method according to claim 24, further comprising causing the compound to
access the stem cells, by administering an effective dose of the compound to the animal by

any of oral, intradermal, subcutaneous, transmucosal, intramuscular or intravenous routes.

26 A method according to claim 2, wherein the compound is functionally equivalent to a protein from the bone marrow morphogenic protein (BMP) family.

. . .

of treating developmental errors in vascular growth or hematopoiesis in an embryo in utero, comprising:
(a) selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and
(b) causing the compound to access a population of embryonic cells in vivo, so as to stimulate the cells to undergo at least one of. . .

28 A method according to claim 27, wherein the compound is an agonist of a hedgehog protein-receptor.

29 A method according to claim 27, wherein the compound is a hedgehog protein.

30 A method according to claim 27, wherein the compound is a first compound capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraernbryonic tissue, so as to enhance the stimulation of hernatopoiesis in. . .

. . .

A method of treating a subject suffering from an abnormal number of erythroid cells, comprising:
(a) selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and
(b) causing the compound to access a population of hematopoietic stem cells over an effective time so as to modulate the number of cells undergoing. . .

32 A method according to claim 3 1, wherein the compound is an agonist of a hedgehog protein-receptor and the hernatopoietic stem cells are stimulated to undergo one of proliferation or hematopoiesis.

33 A method according to claim 32, wherein the compound is a hedgehog protein.

34 A method according to claim 3 1, wherein the compound is a first compound capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraernbryonic tissue, so as to enhance the stimulation of hematopoiesis in. . .

35 A method according to claim 3 1, wherein the compound is an antagonist of a hedgehog protein and the hematopoietic stem cells are inhibited from undergoing one of proliferation or hematopoiesis.

38 A method of treating a subject suffering from an ischernia in tissues,
comprising:
(a) selecting an effective dose of a compound that is functionally
equivalent to a gene product expressed in an extraeffibryonic tissue; and
(b) administering the compound to the ischernic site over an effective time
so as to stimulate vascular growth within the ischernic tissues.

39 A method according to claim 37, wherein the ischerriia is myocardial ischernia.

40 A method according to claim 38, wherein the compound is an agonist of a
hedgehog protein-receptor.

41 A method according to claim 40, wherein the compound is a hedgehog protein.

42 A method according to claim 39, wherein the compound is a first compound
that is capable of acting synergistically with a second compound that is derived from a second
gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of
vascular growth.

43 A method of treating abnormally enhanced vascular growth in a subject,
comprising:
(a) selecting an effective dose of a hedgehog compound capable of
inhibiting the activity of a gene product expressed in an extraembryonic tissue; and
(b) administering the compound to the subject over an effective time so as
to inhibit abnormally enhanced vascular growth.

44 An in vitro assay for determining the activity of a compound capable of
modulating hematopoiesis or vascular growth, comprising:
(a) selecting a population of cells from a tissue derived from a fertilized
egg of. . .

52 An assay for determining the activity of a compound capable of modulating
1 5 hernatopoiesis or vascular growth, comprising:
(a) selecting a first transgenic animal carrying a marker:c-globin hybrid
gene; wherein the. . . animal that is similarly transgenic;
(c) isolating an embryo from the mating during the gestation period; and
(d) determining the effect of the compound on the stimulation of
hernatopoiesis and vascular growth in the isolated embryo by measuring
marker expression.

=>

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